With the author's regards.

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A Case of Compensatory Regulation in the Regeneration of Hydroides dianthus.

By

Charles Zeleny.

With three figures in text.

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A Case of Compensatory Regulation in the Regeneration of Hydroides dianthus.

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In the course of a series of experiments on the regeneration processes of Polychaeta, carried on at the Marine Biological Laboratory at Woods Holl, Mass. during the summer of 1901 I observed a case of regenerative substitution in the common Serpulid, Hydroides, of a kind which seems to be of relatively rare occurrence in animals [1]). It is well known that in this form as in some other Serpulids, the branchial crown, which projects from the opening of the calcareous tube, bears two opercula only one of which is functional, the other being reduced to a rudimentary knob-like structure. As has been frequently pointed out these opercula are undoubtedly modified branchiae. This is indicated not only by similarity in structure and position but also by the character of the opercula in related genera. In Salmacina all of the branchiae have terminal enlargements so that upon retraction the modified ends serve to close up the opening of the tube. In Filograna the opercular structure is confined to two branchiae of equal size which still retain the respiratory filaments. The condition in *Hydroides dianthus* is shown in Fig. 1 (*a*, *b*) and (*d*, *d'*). Fig. 1 (*a*, *b*) is a diagram showing the position of the opercula and branchiae as seen when looking down on the anterior end of the animal. Fig. 1 (*d*) is a side view of the

[1]) I wish to express my great obligation to Professor E. B. Wilson and to Professor T. H. Morgan for much valuable aid and kindly criticism in the conduction of the experiments and the interpretation of the results.

functional operculum of a normal individual. As seen here the operculum consists of a terminal cup or plug and a long stalk. When the animal is active and undisturbed the terminal part of the operculum reaches out to a considerable distance beyond the opening of the tube, giving room for the branchiae to expand and project as two semicircular rows. The terminal cup is double, having two rows of processes, the lower of which are of the nature of serrations while the upper have more the appearance of tentacles but are quite rigid. The stalk shows a distinct suture-like transverse mark near its base. The rudimentary operculum is shown in some of its more common forms in Fig. 1 (*d'*, *e*, *f*). There is usually a slight indication of the division into stalk and cup and sometimes also a circular ridge near the distal end but never in the cases observed any indication of serration of this ridge. The suture-like cross line near the base apparently corresponds to the basal suture of the functional operculum. Here however it is much more marked since the part above the suture is of a pale green color with but slight indications of the dark stripes seen in the basal portion.

An examination of 57 normal individuals gave 31 specimens with a functional operculum on the right side and a rudimentary operculum on the left side while 24 showed the reverse condition, having a functional operculum on the left and a rudimentary one on the right. The remaining two had rudimentary opercula on both right and left sides. In one of these the left one was much larger than the right and was apparently developing into a functional operculum. In the other case the two rudimentary opercula were of equal size.

Diagram I.

Positions of Opercula in Normal Hydroides.

Colony No.	Date	No. of Individuals	R = Right F = Left	R = Left F = Right	R = Right R = Left
I, II, III, IV	IX. 16. 1901	5	3	2	0
V	IX. 19. 1901	15	8	6	1
VI	IX. 19. 1901	37	13	23	1
Total		57	24	31	2

Abbreviations { F = Functional Operculum. R = Rudimentary Operculum.

The experiments performed may be grouped under five heads:

1. The functional operculum was cut off.
2. The rudimentary operculum was cut off.
3. Both functional and rudimentary opercula were cut off.
4. The body of the worm was cut in two in the thoracic region.
5. Observations were made on normal specimens which were taken out of their tubes but were not otherwise operated on.

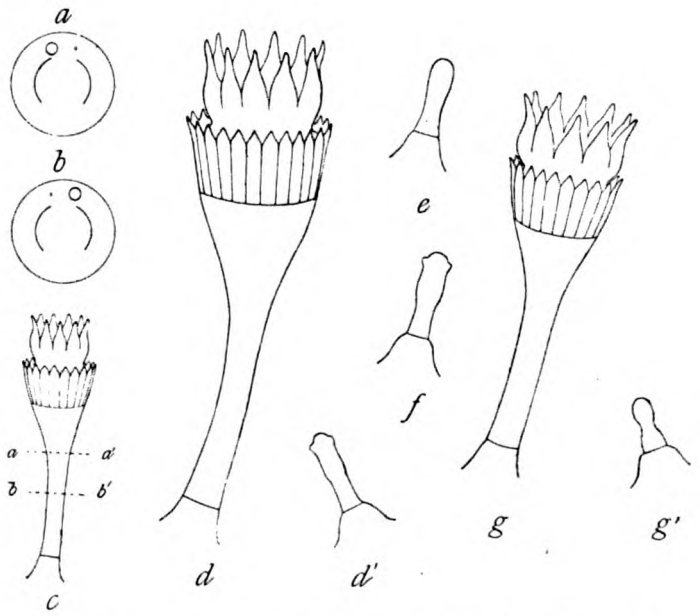
All of the worms used in these experiments were taken out of their tubes and after the operation were placed in dishes of sea-water. No new tubes were secreted, so that the regeneration processes described took place without any external stimulus due to their presence.

1. Operation on the Functional Operculum. In this series of experiments the stalk of the functional operculum was cut transversely in the region indicated in Fig. 1 (*c*), between the lines *aa'* and *bb'*. This operation left a considerable part of the stalk still attached to the animal. In all, 23 worms were operated on in this way. Eighteen of these (Set 1) were kept for 22 days after the operation and the other five (Set 2) for only four or five days.

In the first set, five had died before the end of the 22 days. The remaining thirteen, with one exception, showed that the rudimentary operculum had developed so as to resemble the original functional operculum in size and all other characteristics (see Fig. 1 [*g*]). The stalk of the original functional operculum, which immediately after the operation still remained attached to the animal, was found in all these cases to have been broken off at the basal suture leaving a small stump. From the distal end of this stump a bud-like outgrowth had taken place, resembling the original rudimentary operculum (see Fig. 1 [*g'*]). The falling off of the stalk at the basal suture is of interest because attempts to pull off or break off the functional opercula of normal individuals proved futile in all cases. When the operculum was seized with a pair of forceps no amount of jerking could break the stalk. The same was found to hold true of the stalk immediately after an operation in which the terminal cup of the operculum had been removed. We may therefore conclude that the cutting of the stalk of the functional operculum inaugurates changes which lead, first, to the development of the rudimentary operculum into a

functional one and, second, to the weakening of the old stalk at the basal suture causing it to drop off. This last result allows the development of a new rudimentary operculum from the stump of the old functional one. The time at which the basal suture is completely loosened varies in different cases. An observation made seven days after the operation showed that one of the Hydroides still had the old functional stalk attached to the body

Fig. 1.



Hydroides dianthus. *a*. Diagram of anterior end showing the relations of opercula and branchiae. *b*. The opercula in the opposite position to those of *a*. *c*. Functional operculum showing position of transverse cuts (× 9). *d*, *d'*. Functional and rudimentary opercula in normal condition. Both from same individual (× 17). *e*, *f*. Typical rudimentary opercula from two normal individuals (× 17). *g*, *g'*. Opercula 22 days after transverse section of stalk of functional operculum. *g*. New functional operculum developed from old rudimentary one. *g'*. New rudimentary operculum developed from stump of old functional one (× 17).

while the former rudimentary operculum was two-thirds developed. This case is represented in Fig. 2 (*a*, *a'*). All the others in the set had at this time lost the old stalk.

As mentioned above there was one exception to the general rule as regards the change of the rudimentary to a functional and the functional to a rudimentary operculum. In this case at the end of 22 days both opercula were well developed although one was considerably larger than the other.

Diagram II.

Operation on Functional Operculum (Set 1).

Number of Specimens	Date of Operation	Date of Observation	Time in Days	Number Dead	$F_1 = R_2$ $R_1 = F_2$	$F_1 = F'_2$ $R_1 = F''_2$
7	VIII. 31. 1901	IX. 22. 1901	22	3	4	—
2	IX. 1. 1901	IX. 23. 1901	22	1	1	—
9	IX. 1. 1901	IX. 23. 1901	22	1	7	1
18				5	12	1

Abbreviations
F_1 = Original Functional Operculum.
R_1 = Original Rudimentary Operculum.
F_2 = Resulting Functional Operculum.
R_2 = Resulting Rudimentary Operculum.

The five specimens grouped together as set 2 were kept under observation for only a few days. Two of them were observed five days after the operation and each showed the former rudimentary operculum well started in its further development, but there was a difference as regards the old functional stalk. This stalk was already detached in one specimen and a new bud was growing from the distal end of the stump but in the other case the old stalk remained attached as before the operation. The former condition is represented in Fig. 2 (*b*, *b'*).

The other three of the above five Hydroides were observed at the end of four days and all showed the former rudimentary operculum with a good start in development. The old functional stalk had broken off at the basal suture in all three cases and a new bud had started from the distal end of the stump. Fig. 2 (*c*, *c'*) gives the regenerating opercula of one of the specimens.

Diagram III.

Operation on Functional Operculum (Set 2).

Number of Specimens	Date of Operation	Date of Observation	Time in Days	Dead	$F_1 = R_2$ $R_1 = F_2$	$F_1 = S$ $R_1 = R_2$
1	IX. 19. 1901	IX. 24. 1901	5	0	1	—
1	IX. 19. 1901	IX. 24. 1901	5	0	—	1
3	IX. 20. 1901	IX. 24. 1901	4	0	3	—
5				0	4	1

Abbreviations
See Diagram II.
($F_1 = S$) indicates that the old Functional Stalk had not dropped off.

2. Operation on the Rudimentary Operculum. In this set of experiments the rudimentary operculum was cut off as near the base as possible. Seven specimens were operated upon. In all of these the rudimentary operculum had begun to regenerate at the end of four days (3 specimens) or six days (4 specimens). The functional operculum remained unchanged. Unfortunately these experiments were begun so near the end of my stay at Wood's Holl that it is impossible to say whether the regenerating bud would have remained as a rudimentary operculum or whether it would have developed into an operculum similar to the functional one. Fig. 2 (*d*) represents one of the cases just mentioned.

Diagram IV.

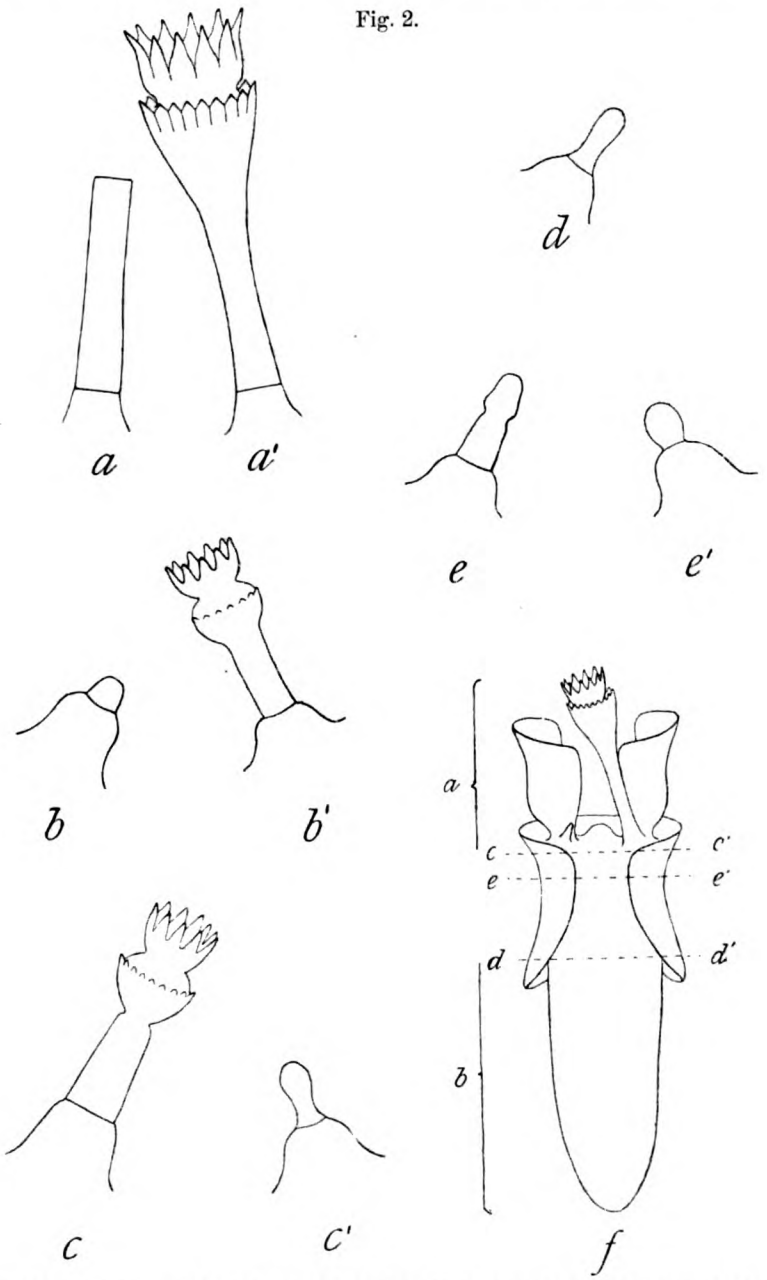
Operation on Rudimentary Operculum.

Number of specimens	Date of Operation	Date of Observation	Time in Days	R_1 Regenerating F_1 Unchanged
4	IX. 17. 1901	IX. 23. 1901	6	4
3	IX. 20. 1901	IX. 24. 1901	4	3
Total 7				Total 7

Abbreviations { See Diagram II.

3. Operation on both the Functional and the Rudimentary Operculum. Under this heading are included the cases in which both the functional and the rudimentary operculum were cut off at the same time. The functional operculum was cut off at about the middle of the stalk and the rudimentary operculum near the base. Six specimens were operated on. One of these had died at the end of six days and in the other five the rudimentary operculum showed in each case a distinct developing bud. In four the old functional stalk had dropped off, breaking at the basal suture, and a bud was growing from the distal end of the stump (Fig. 2 *e*, *e'*). In the fifth case the old stalk had not yet dropped off. In none was the time long enough to determine the final disposition of the growing buds. The original rudimentary opercula were the first to start to regenerate and at the end of six days were larger than the regenerating functional ones. One of the former showed slight indications of serrations near the terminal end of the growing knob indicating that it would probably have developed into a functional operculum.

Fig. 2.



Hydroides dianthus. *a*, *a'*. Opercula seven days after transverse section of stalk of functional operculum. *a*. Former functional operculum. *a'*. Former rudimentary operculum (× 25). *b*, *b'*. Same opercula as shown in Fig. 1 *d*, *d'* but five days after transverse section of stalk of functional operculum. *b*. Former functional operculum. *b'*. Former rudimentary operculum (× 25). *c*, *c'*. Opercula four days after transverse section of stalk of functional operculum. *c*. Former rudimentary operculum. *c'*. Former functional operculum (× 25). *d*. Regenerating rudimentary operculum four days after operation upon it (× 25). *e*, *e'*. Opercula four days after both had been operated on. *e*. Former rudimentary operculum. *e'*. Former functional operculum (× 25). *f*. Diagram of dorsal view to show position of cuts.

Diagram V.

Operation on Both Opercula.

Number of Specimens	Date of Operation	Date of Observation	Time in Days	Dead	$F_1 = r'$ $R_1 = r''$	$F_1 = S$ $R_1 = r$
1	IX. 17. 1901	IX. 23. 1901	6	—	—	1
5	IX. 20. 1901	IX. 26. 1901	6	1	4	—
Total 6				1	4	1

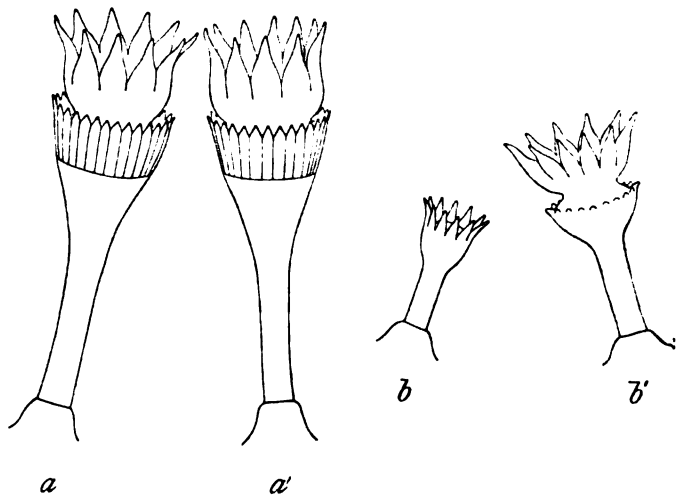
Abbreviations { See Diagrams II and III.
r, r', r'' = developing bud-like Opercula.

4. Operations in which the Animal was divided into two Parts by means of a Transverse Cut in the Thoracic Region. The transverse cuts were made in the region indicated in Fig. 2 (*f*), by the area included between the lines *cc'* and *dd'*. The line *ee'* represents the place at which the great majority were divided and is so situated as to include about three thoracic segments with the anterior piece (*a*), the remainder going with the posterior piece (*b*). In the present paper we are concerned only with the changes that take place at the anterior end of the posterior piece *b*. The level at which the division was made had a great influence upon the rapidity of regeneration. If, for instance, the cut surface was near the anterior end in the neighborhood of *cc'* the process of replacement of the lost parts progressed much more rapidly than if the cut was in the region *dd'*. On this account, and because of other differences in the conditions, there was a great variety in the stages of development in which the regenerating opercula were found. Since it is necessary for our purpose to distinguish between the opercula that are still in the rudimentary stage and those which have grown beyond this stage, and since in the 57 normal specimens examined there were none with the rudimentary operculum showing any distinct circle of projections or serrations near its distal end, the presence or absence of such circle of projections may be taken as a diagnostic character. All those with the circle of projections will be classed as functional opercula and those without it as rudimentary ones. This arbitrary line of separation is of value because all those that have developed beyond the point named may be considered as probably on the way to full development.

In the large majority of the cases the regenerating opercula did not show the normal differentiation into a functional and a rudimentary one, but two fully developed

opercula resulted. The accompanying diagram (Diagram VI) gives a summary of the experiments. It shows that out of the 72 specimens operated on, 29 were alive at the end of 21 days. Of these eighteen showed both opercula developed beyond the rudimentary stage. Fig. 3 (*a*, *a'*) represents one of these cases in which both opercula were fully developed and Fig. 3 (*b*, *b'*) another in which the two were of unequal size and not fully developed. In five both opercula were

Fig. 3.



Hydroides dianthus. *a*, *a'*. Two opercula as developed upon the anterior end of the posterior piece after division by a transverse cut in the thoracic region. Twenty-two days after operation (× 17). *b*, *b'*. Opercula of unequal size as developed upon the anterior end of a posterior piece eleven days after the operation (× 17).

still rudimentary and in five others one operculum was in the functional stage and the other in the rudimentary stage. In a single case there was a rudimentary operculum on one side and no operculum on the other. This last specimen showed no branchiae on the side where the operculum was absent.

5. Observation of Normal Specimens to ascertain Presence or Absence of Progressive Changes. Several normal specimens were observed at intervals for ten days in order to ascertain whether there were any progressive changes in the opercula. In this instance the Hydroides were taken out of their tubes but were not otherwise disturbed. No changes of any kind were noticed in the opercula.

Diagram VI.

Transverse Division of Body in Thoracic Region.

Register Number	Number of Spec.	Date of Observation	Date of Operation	Time in Days	Dead	F + F	R + R	F + R	R + —
140	6	VIII. 23. 1901	IX. 21. 1901	29	6	—	—	—	—
141	6	VIII. 23. 1901	IX. 21. 1901	29	5	1	—	—	—
142	9	VIII. 23. 1901	IX. 21. 1901	29	9	—	—	—	—
177—201	25	VIII. 31. 1901	IX. 21. & 22. 1901	21—22	11	9	1	3	1
203	26	IX. 1. 1901	IX. 22. 1901	21	12	8	4	2	—
Total	72				43	18	5	5	1

Abbreviations:
F + F = Both Opercula in Functional Stage.
R + R = Both Opercula in Rudimentary Stage.
F + R = One Operculum in Functional and one in Rudimentary Stage.
R + — = Only one Operculum present and that a Rudimentary One.

Compensatory regeneration of the type here described seems to be of relatively rare occurrence though compensatory hypertrophy has long been a familiar fact in the case of bilateral organs, such as the lung or kidney after the removal or destruction of the organ on one side. In such cases the usually accepted explanation is that hypertrophy of the remaining organ is due to increased functional activity; but it would be difficult to explain the case here described on so simple an hypothesis. It corresponds more nearly with the case described by PRZIBRAM[1]) in the Crustacean, Alpheus. In this case the two anterior claw-bearing legs differ both in size and structure. When the larger is cut off, the smaller at the first or second moult assumes the character of the larger one. The stump of the removed leg upon regeneration acquires the character of the former smaller one; so that we have a result very similar to that given above for the corresponding experiment on the opercula of Hydroides.

It is quite clear that the rudimentary operculum is a structure whose power of development is held latent under ordinary conditions. It seems equally clear that it is a vestigial organ derived from a functional ancestral type. The most obvious explanation would seem to be that its presence is an adaptation to provide for as rapid as possible a replacement of the functional one after loss. The difficulty

[1]) PRZIBRAM, Experimentelle Studien über Regeneration. Archiv f. Entwickelungsmechanik. Bd. XI. 1901.

experienced in artificially tearing off the latter however indicates that loss due to injury must be of rare occurrence. A periodic loss due to organic change in the operculum itself is not thereby excluded, though observations during a limited time showed no sign of such a change. Facts show that under normal conditions a state of equilibrium exists when one large and one small operculum are developed and the presence of the former directly or indirectly inhibits the power of development of the latter. During regeneration the restraining influence is removed and the rudimentary operculum having already as it were accomplished a part of its development immediately goes forward to complete the process; the development of the anlage from the base of the old operculum being now in its turn inhibited by the presence of the larger one. This is of course not an explanation but only a restatement of the facts. The statement is however suggestive when the facts are compared with those occurring in the regeneration of the entire head, where, as described above, two functional opercula are developed. The equal size of the opercula in this case may be due merely to the fact that they develop at the same time; but whether this explanation is adequate cannot be proved until we know how the opercula develop in the embryo. It seems not impossible on the other hand that the failure to establish the normal equilibrium in the regeneration of decapitated worms may be due to the fact that the cerebral ganglia are absent or in an undeveloped state while the opercula are forming. Further study is required to throw light on this question, but it is perhaps worth while to point out that the regeneration of two equal functional opercula in the decapitated worms shows some of the features of a reversion to the probable ancestral type with two functional opercula (as in Filograna) though the regenerated ones show no branchial filaments.

Summary.

The results obtained may be grouped as follows:

1) In normal specimens of *Hydroides dianthus* there is one functional and one rudimentary operculum. The functional may be on the right side and the rudimentary on the left or vice versa.

2) When the stalk of the functional operculum is cut in two distal to the basal suture, the rudimentary operculum begins to develop very soon and finally reaches the size of the former functional

one. On the other hand, the part of the stalk of the old functional operculum, which immediately after the operation still remains attached to the body, drops off in a few days and a bud-like outgrowth takes place from the distal end of the stump. This bud however soon stops its development, remaining as the new rudimentary operculum. Thus an interchange of position is the final result of the operation.

3) When the rudimentary operculum is cut off near its base, there is no noticeable effect on the functional one. The rudimentary operculum starts to regenerate very soon after the operation.

4) When both the functional and rudimentary opercula are cut off, the stalk of the functional one breaks off at the basal suture as in the former case where only the functional was operated on, and new opercula-buds start to develop on both sides. Four days after the operation the buds on the rudimentary side are larger than those on the functional side.

5) When the body of the animal is cut in two in the thoracic region the regeneration at the anterior end of the posterior piece shows that in the majority of the cases the tendency is to produce two fully developed opercula, each resembling the original functional one.

6) Normal specimens show no progressive changes in the opercula.

Department of Zoology, Columbia University, Oct. 18th, 1901.

Zusammenfassung.

Die erhaltenen Resultate können in folgenden Sätzen zusammengefasst werden:

1) Normale Exemplare von *Hydroides dianthus* besitzen ein funktionirendes und ein rudimentäres Operculum. Das funktionirende kann sich auf der rechten, das rudimentäre auf der linken Seite befinden oder umgekehrt.

2) Wenn der Stiel des funktionirenden Operculums distal von der Basalnaht entzwei geschnitten wird, fängt das rudimentäre Operculum sehr bald an sich zu entwickeln und erreicht schließlich die Größe des funktionirenden. Andererseits verschwindet der unmittelbar nach der Operation noch am Körper gebliebene Stielrest des Operculums im Verlauf einiger Tage und am distalen Ende des Stumpfes entsteht ein knospenähnlicher Auswuchs. Jedoch steht die Entwickelung dieser Knospe bald still, indem sie auf dem Stadium des neuen rudimentären Operculums jetzt ihrerseits verbleibt. Auf diese Weise besteht das schließliche Operationsresultat in einem Stellungsaustausch der beiden Opercula.

3) Wird das rudimentäre Operculum nahe seiner Basis abgeschnitten, so hat das keinen merklichen Einfluss auf das andere, funktionirende. Das rudimentäre Operculum beginnt sich sehr bald nach der Operation zu regeneriren.

4) Schneidet man beide, das funktionirende und das rudimentäre Operculum ab, so schlägt der Stengel des funktionirenden an der Basalnaht aus wie im vorigen Fall, in dem nur an dem funktionirenden operirt wurde, und neue Operculumknospen beginnen an beiden Seiten ihre Entwickelung. Vier Tage nach der Operation sind die Knospen an der Seite des ehemaligen rudimentären Operculums größer als die auf der Seite des früheren funktionirenden.

5) Wird der Körper des Thieres in der Thoraxregion durchschnitten, so zeigen die am Vorderende des hinteren Stückes auftretenden Regenerationsvorgänge, dass in der Mehrzahl der Fälle die Tendenz zur Hervorbringung zweier vollentwickelter Opercula besteht, von denen jedes dem ursprünglich funktionirenden ähnelt.

6) Normale Exemplare zeigen keine fortschreitenden Veränderungen bezüglich ihrer Opercula.